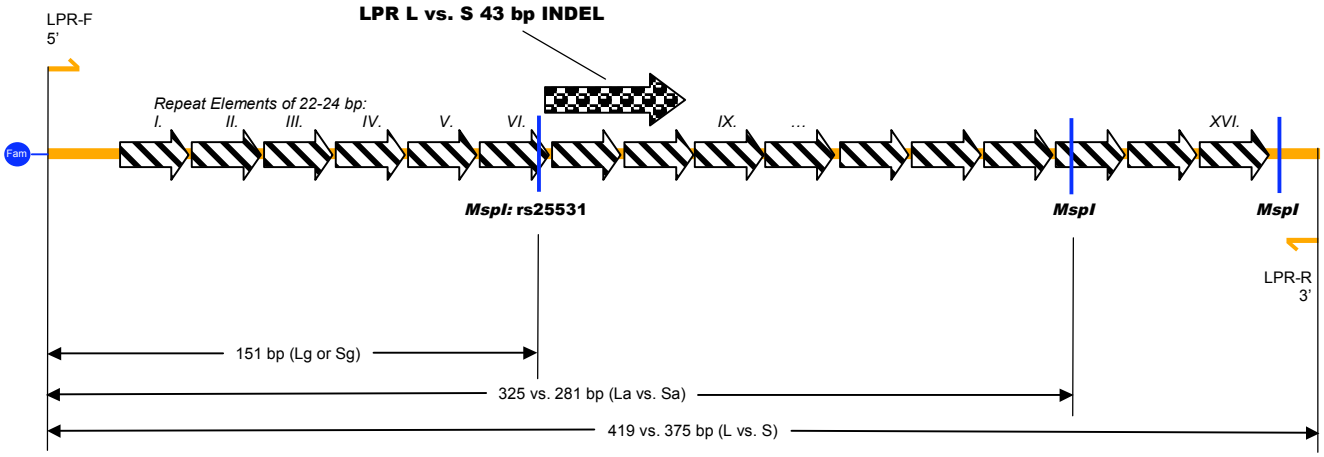
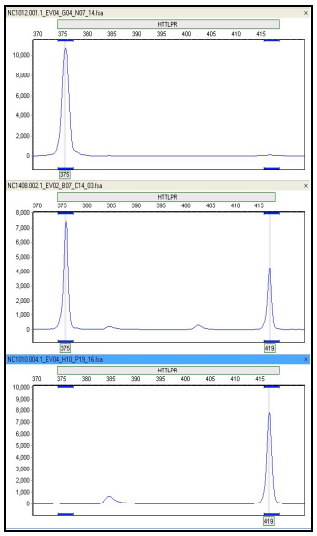
Online supplemental figure 2: *SLC6A4* gene-linked polymorphic region (LPR) *MspI* Digest Map and 2-step protocol for genotyping rs25531

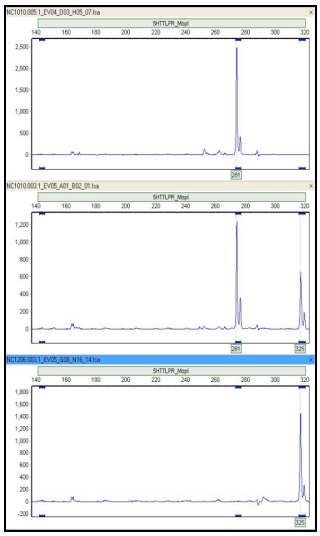


Step I: **PCR amplification of LPR L and S alleles**

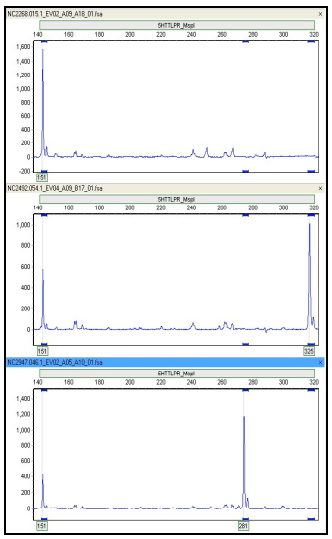


S/S (371 bp), top
L/S (419/371 bp), middle
L/L (419 bp), bottom

Step II: ***MspI*** **Digestion of LPR amplicons from step I**



Sa/Sa (281 bp), top
La/Sa (325/281 bp), middle
La/La (325 bp), bottom



Sg/Sg, Lg/Sg, Lg/Lg (151 bp), t
La/Sg, La/Lg (325/151 bp), m
Sa/Sg, Lg/Sa (151/281 bp), b

Methods: Phase-certain haplotyping of the LPR variants La, Lg, Sa and the rare Sg allele was performed by a 2-step protocol: I. amplification of the LPR for determination of S or L allele; and, II. digestion of this amplicon with *MspI* restriction endonuclease. A constant *MspI* digest site present within the 419-bp amplicon served as internal control for digestion or partial digestion. Step II results (rs25531) were obtained only after unambiguous genotyping in step I. Separation of the digestion products by capillary electrophoresis and analysis (Gene Marker v1.5) allowed heuristic assemblage of genotypes generated by steps I and II. This resulted in the following restriction fragment configurations (bp): SS (281), SLa (281/325), SLg (281/151), LaLa (325), LaLg (325/151), and LgLg (151). Only *MspI* activity at the variable *MspI* site resulted in a 151-bp fragment. A rare Sg FAM-labeled 151-bp fragment obtaining only from the S allele was detected and confirmed by sequencing. All genotypes were then assessed for discordance and Mendelization-type errors. Step I vs. step II genotype completion rates: 99.6 vs. 96.5%, respectively.